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Modifying effects of depression on the association between *BDNF* methylation and prognosis of acute coronary syndrome

Running title: Depression and *BDNF* methylation on prognosis of ACS

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Abstract

Aims: Brain-derived neurotrophic factor (BDNF) plays important roles in angiogenesis, inflammation, and neuronal plasticity. BDNF methylation has been extensively investigated in depression, but not in cardiac diseases. We asked whether BDNF methylation status is associated with a major adverse cardiac event (MACE), inflammation, and the association with depression comorbidity and its treatment in patients with acute coronary syndrome (ACS).

Methods and results: A cross-sectional baseline study and nested 24 week double-blind escitalopram placebo-controlled trial (ClinicalTrial.gov identifier NCT00419471) were performed from 2006 to 2012, with 5–12 year follow-up for MACE. Patients with recent ACS (969 total) were divided into four groups according to depression comorbidity at baseline and treatment allocation: 591, absent depression; 127, depression on escitalopram; 128, depression on placebo; 123, depression on care as usual (CAU). BDNF methylation was measured in leucocyte DNA, and multiple demographic and clinical characteristics including interleukin 6 were evaluated as covariates at baseline. The primary outcome, time to first MACE (a composite of all-cause mortality, myocardial infarction and percutaneous coronary intervention), was investigated using Cox regression models after adjustment for covariates. Interleukin 6 level was significantly higher in patients with higher BDNF methylation values. Higher BDNF methylation was associated with increased MACE independent of confounding factors [HR (95% CI) = 1.45 (1.17-1.78)]. This association was significant in patients without depression [HR (95% CI) = 1.39 (1.01-1.90)] and depressive patients on placebo [HR (95% CI) = 1.72 (1.02-3.02)] or CAU [HR (95% CI) = 1.53 (1.01-2.61)], but not in those treated with escitalopram [HR (95% CI) = 1.00 (0.51-1.95)].

Conclusion: BDNF methylation was significantly associated with prognosis of ACS.

Escitalopram may mitigate the deleterious effect of higher BDNF methylation in depressive patients with ACS. Further research is needed to elucidate the mechanistics and to assess the generalisability of these findings.

Clinical Trial: ClinicalTrial.gov registry number for 24week trial: NCT00419471

Keywords: acute coronary syndrome; epigenetic methylation; BDNF; depression; cohort study

Abbreviations: BDNF, brain-derived neurotrophic factor; MACE, major adverse cardiac event; ACS, acute coronary syndrome; CAU, care as usual; DNA, deoxyribonucleic Acid; IL, interleukin; ESR 1, oestrogen receptor 1; ABCG, ATP-binding cassette subfamily G member; *FOXP3,* forkhead box P3; *F2RL,* F2R-like thrombin or trypsin receptor; K-DEPACS, Korean DEPression in Acute Coronary Syndrome; EsDEPACS, Escitalopram for DEPression in Acute Coronary Syndrome; CNUH, Chonnam National University Hospital; KAMIR, Korea Acute Myocardial Infarction Registry; MI, myocardial infarction; MINI, Mini-International Neuropsychiatric Interview; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; mRNA, messenger ribonucleic Acid; PCR, polymerase chain reaction; BDI, Beck Depression Inventory; HAMD, Hamilton Depression Rating Scale; PCI, percutaneous coronary intervention; HR, hazard ratio; CI, confidence interval.

Introduction

Acute coronary syndrome (ACS) is the leading cause of mortality and disability worldwide (GBD 2016 DALYs and HALE Collaborators, 2017). Multiple biochemical markers are involved in the prognosis of ACS (Panteghini, 2004). Recently, several lines of evidence have indicated that epigenetic factors play key roles in the development of atherosclerosis, the main underlying mechanism of ACS (Grimaldi et al., 2015). 'Epigenetics' refers collectively to alterations in gene expression not associated with changes in sequence or responses to environmental stimuli (Bernstein et al., 2007). DNA methylation, one of the most well-known epigenetic modifications, entails covalent methylation of the C5 position of cytosine residues followed by guanine residues (CpG dinucleotides) (Wolffe et al., 1999).

Most previous studies of the association between DNA methylation status in candidate genes and ACS employed a case-control design. Hypermethylation in the promoters of *ESR1* (oestrogen receptor 1) (Huica et al., 2011), *ABCG1* (ATP-binding cassette subfamily G member 1) (Peng et al., 2014) and *FOXP3* (forkhead box P3) (Jia et al., 2013), and hypomethylation in the promoter of *IL6* (interleukin 6) (Yang et al.,2016), are associated with ACS. A few studies have investigated this issue in a high-risk population of patients with ACS from the standpoint of risk of recurrence or death. The results revealed that hypomethylation of *F2RL3* (F2R-like thrombin or trypsin receptor 3) is associated with increased mortality in ACS patients (Breitling et al., 2012).

Epigenetic modification of the gene encoding brain-derived neurotrophic factor (BDNF) has been implicated in ACS. BDNF enhances vascular flow and promotes revascularisation of ischemic tissue (Donovan et al., 2000; Kermani et al., 2005). Notably in this context, higher serum BDNF is associated with decreased risk of CVD and mortality (Kaess et al., 2015). *BDNF* expression is regulated by an epigenetic mechanism; i.e.,

hypermethylation of the *BDNF* promoter is associated with reduced synthesis of BDNF (Martinowich et al., 2003). Therefore, *BDNF* hypermethylation may be associated with poor prognosis of ACS by increasing morbidity or mortality.

BDNF hypermethylation has also been associated with elevated risk of depressive disorder (Fuchikami et al., 2011; Kim et al., 2013) and better response to antidepressant treatment (Tadić et al., 2014; Kim et al., 2015a), both in general and ACS populations. In addition, comorbidity and treatment of depression have affected cardiac outcomes in patients with ACS (Lespérance et al., 2002; Kim et al., 2018a). These observations beg the question of whether depression may have a role on the association between *BDNF* methylation status and prognosis of ACS.

We previously reported that higher *BDNF* methylation status was independently associated with depressive disorder in 969 patients with recent ACS (Kim et al., 2015b). By extending the cohort and to address these unanswered questions, we sought to investigate whether *BDNF* methylation status affects the long-term prognosis of ACS and examine whether these effects differed according to depression comorbidity and treatment status.

Methods

Study outline and participants

The analyses described in this study were carried out using data from a prospective observational study of patients with ACS, Korean DEPression in ACS (K-DEPACS), which also included a nested randomised clinical trial (RCT) for patients with depression and ACS, Escitalopram for DEPression in ACS (EsDEPACS). The design and main findings of K-DEPACS and EsDEPACS have been published (Kim et al., 2018a; Kim et al., 2018b), and the eligibility criteria are described in the online supplementary material. This investigation conformed to the principles outlined in the Declaration of Helsinki. Written informed consent was collected for both studies, which were approved by the Chonnam National University Hospital (CNUH) Institutional Review Board. The outline and participant recruitment process for analysis performed in this study are presented in Figure 1.

K-DEPACS baseline evaluation

From 2006 to 2012, participants were consecutively recruited from patients recently hospitalised with ACS at the Department of Cardiology, CNUH, Gwangju, South Korea. This department was nominated by the Korean Circulation Society to serve as the central coordinating centre for the Korea Acute Myocardial Infarction Registry (KAMIR) (Lee et al., 2011). KAMIR is a nationwide prospective, multicenter, online registry (http://kamir5.kamir.or.kr/) designed as a surveillance platform to track clinical outcomes of patients with acute MI without exclusion criteria to reflect real-world practice; this enables prospective associations to be evaluated for a range of exposures or interventions with long-term cardiac outcomes. Patients were treated by cardiologists participating in the study based on international guidelines for the management of ACS (Anderson et al., 2012). Those who

met eligibility criteria and agreed to participate were selected for K-DEPACS. All baseline evaluations, including depression, were made in a hospital within 2 weeks of the ACS episode. Diagnoses of depressive disorder were determined by psychiatrists using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998), a structured diagnostic psychiatric interview for disorders in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994), defining major or minor depressive disorder categories as outputs. According to these criteria, patients were diagnosed as having major depressive disorder if they had at least one core symptom (i.e., depressed mood or loss of interest) and at least four other symptoms of depression, and as having minor depressive disorder if they had at least one core symptom and at least two but fewer than five total symptoms.

BDNF methylation status was examined in patients who agreed to offer blood samples. DNA was extracted from venous blood using standard procedures. The *BDNF* region chosen for methylation analysis is a CpG-rich area (containing nine CpG sites) in exon VI that also serves as the promoter region; it is located from nucleotides -612 to -463 relative to the transcriptional start site in exon VIII (online Figure S1). These data have been deposited in GenBank (accession number: BankIt1568919 *BDNF* JX848620). This region was chosen because it corresponds to an analogous region of rat *BDNF* that is differentially methylated and associated with *BDNF* mRNA expression and because it has been investigated in the context of antenatal depression (Roth et al., 2009; Devlin et al., 2010).

Genomic DNA (1 μg) was extracted from leucocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The DNA was then subjected to bisulphite treatment using the EpiTech Bisulfite Kit (Qiagen). A 150 bp fragment of the *BDNF* promoter was amplified by polymerase chain reaction (PCR) from bisulphite-treated DNA using the forward (5'-GTGGGGTAGGAGGGGAGTAGTAT-3') and reverse (5'-

AAATCCCCCAATCAACTCTCT-3') primers. PCR conditions were as follows: 95°C for 15 minutes, followed by 45 cycles of 95°C for 15 seconds, 57°C for 30 seconds and 72°C for 15 seconds, with a final extension of 5 minutes at 72°C. PCR products were sequenced using the PSQ 96M Pyrosequencing System (Biotage) using the primer 5'-GGTAGGAGGGGAGTAGTA-3'. The methylation percentage at each CpG region was quantified using the Pyro Q-CpG software, version 1.0.9 (Biotage). Individual methylation percentages at the remaining six CpG sites and their average values were used in the analyses. Because the methylation percentage at CpG sites 2, 6 and 8 was 100% in all participants, these three sites were excluded.

Information was collected regarding characteristics that could potentially affect cardiac outcomes (Jaffe et al., 2006). Demographic data were obtained on age, gender, education, marital status, living alone, housing and employment status. For depression characteristics, the self-completed Beck Depression Inventory (BDI) (Beck et al., 1961) score and previous or family history of depression were recorded. The following cardiovascular risk factors were ascertained: diagnosed hypertension and diabetes mellitus, hypercholesterolemia by fasting serum total cholesterol level (>200 mg/dL), obesity (based on measured body mass index), reported current smoking status, and previous and family histories of ACS. Cardiac severity status was identified: ACS diagnosis (MI or unstable angina), Killip classification (Killip et al., 1967), left ventricular ejection fraction and serum levels of troponin I and creatine kinase-MB. Since pro-inflammatory cytokines reduced the BDNF gene expression (Lapchak et al., 1993), serum IL6 level was also measured.

The nested EsDEPACS study

Of patients with baseline diagnoses of depressive disorder, those who met eligibility criteria and agreed to participate were randomised into a 24 week, double-blind, placebo-controlled

trial of escitalopram, the EsDEPACS study (ClinicalTrial.gov registry number: NCT00419471). The first patient was enrolled in May 2007, and the last patient completed follow-up evaluation in March 2013. Examinations were scheduled at baseline, and in weeks 4, 8, 12, 16, 20 and 24 thereafter. The primary efficacy measure was the Hamilton Depression Rating Scale (HAMD) (Hamilton et al., 1960). The details and main results of this trial have been published previously (Kim et al., 2015a): escitalopram was superior to placebo in both primary depressive and secondary outcomes. The remaining participants who did not meet the eligibility criteria or declined participation in the trial received conventional medical treatment for ACS only.

Long-term cardiac outcomes

Comprehensive evaluations for cardiac outcomes are possible for this study because KAMIR manages and records detailed electronic data on hospital admissions, deaths, recurrent myocardial infarction (MI) and percutaneous coronary intervention (PCI). All baseline participants were successfully followed up for these outcomes. To enable non-hierarchic endpoint analyses, all patients were followed for the evaluation point of interest or until death. The primary endpoint was a major adverse cardiac event (MACE), which was a composite of all-cause mortality, MI and PCI. Secondary endpoints were all-cause mortality, cardiac death (defined as sudden death when no other explanation was available, death from arrhythmias or after MI or heart failure, or death caused by heart surgery or endocarditis), MI and PCI. An independent endpoint committee composed of study cardiologists adjudicated all potential events and was blinded to the participants' depression comorbidity.

Statistical analyses

According to their depression comorbidity and treatment status at baseline, the patients were

divided into four groups: absent depression, depression on escitalopram, depression on placebo and depression on care as usual (CAU). Baseline demographic and clinical characteristics were compared between the four groups by analysis of variance or $\chi 2$ tests with post-hoc comparisons. *BDNF* methylation percentages were categorised by a binary variable with value 'lower' or 'higher' (i.e., below or above median value), arbitrarily but in keeping with previous research (Breitling et al., 2012). Baseline characteristics were compared again between patients with lower and higher average *BDNF* methylation values using t-tests or χ^2 tests, as appropriate. Characteristics significantly associated with average *BDNF* methylation values ($p<0.05$) and other variables with potential effects on MACE were used as covariates in further adjusted analyses. Kaplan–Meier models were used to compare the cumulative proportion of participants experiencing composite and individual MACE (defined according to the date of the first event for each patient) between those with lower and higher *BDNF* methylation values. Cox proportional hazards models were used to compare time to first composite and individual MACE, after adjustment for the potential covariates described above, between the two groups. To investigate the extent of mediating effects of baseline characteristics cluster on the associations between average *BDNF* methylation values and MACE outcomes, Cox proportional hazards models were repeated by adjusting the characteristics cluster on socio-demographics, depression, cardiac risk factors, and current cardiac status, separately. To evaluate the differences in effects of average *BDNF* methylation values on composite and individual MACE, the Cox proportional hazards models were used in these four groups with calculating interaction terms after adjustment for all the potential covariates. Additional sensitivity analyses were conducted using the methylation value as a continuous exposure variable (10 percent unit increase) to reexamine its effect beyond the binary categorical approach. All statistical tests were two-sided with a significance level of 0.05. Statistical analyses were carried out using the SPSS 21.0 and

1 STATA 12.0 software.

Results

Recruitment

The recruitment process is described in Figure 1. Of 4809 patients with ACS, 1152 were recruited, of whom 969 (84%) agreed to provide blood samples. Those who did or did not agree to provide blood samples did not differ significantly with respect to any baseline characteristic (all p-values >0.15). Of these 969 participants, depressive disorder at baseline was absent in 591 and present in 378 (39%). Of those with depressive disorder, 255 participated in the EsDEPACS trial (127 randomised to escitalopram and 128 to placebo), and 123 received CAU. Baseline characteristics are described for these four groups in online Table S1. Significant group differences were found in gender, marital status, living alone, housing, employment status, BDI scores, DSM-IV diagnosis of depressive disorder, hypertension, diabetes, smoking status, and ACS diagnosis. In particular, the two treatment allocated groups had more severe depression pathologies than the CAU group. All participants were followed for cardiac outcomes over 5–12 years, until 2017 or their death [median; mean (standard deviation) duration of follow-up = 8.4; 8.7 (1.5) years].

BDNF methylation status and baseline characteristics

Median (interquartile rage) and mean (standard deviation) percentages of average *BDNF* methylation and methylation at six individual CpG sites are described in online Table S2. The methylation percentages of the six CpG sites were highly correlated (online Table S3). Given these high correlations, the results of the following analyses were robust for average CpG values and similar but less obvious for individual CpG sites. Therefore, we only present the results for average CpG values in the main manuscript; those for individual CpG values are reported in the online supplementary material. In Table 1, baseline characteristics are

compared between subjects with lower and higher average *BDNF* methylation values. A higher average BDNF methylation value was significantly associated with unmarried marital status, unemployment, higher BDI scores, depression comorbidity and treatment, family history of depression and, lower LVEF., and higher serum IL-6 levels. These characteristics were included as covariates in subsequent analyses (except for depression comorbidity and treatment, to avoid collinearity with BDI scores). In addition, age, education, all cardiac risk factors and current cardiac status variables were also considered as covariates because they have been associated with cardiac outcomes in previous studies (Panteghini. et al., 2004; Jaffe et al., 2006).

Effects of average *BDNF* methylation values on the occurrence of MACE

The primary endpoint (composite MACE) occurred in 383 participants (39.5%), and secondary endpoints occurred in 178 (18.4%) for all-cause mortality, 98 (10.1%) for cardiac death, 101 (10.4%) for MI and 139 (14.3%) for PCI. Figure 2 illustrates cumulative risk of the composite MACE in subjects with lower and higher average *BDNF* methylation. A significant difference was observed: composite MACE incidences were 33.1% (159/481) in lower and 45.9% (224/488) in higher [hazard ratio (HR), 1.45 (95% CI, 1.17–1.78); p-value=0.001]. Figure 3 illustrates cumulative risks of individual MACE components in the two groups. Significant differences were observed in the incidences of all-cause mortality [15.0% in lower and 21.7% in higher (HR, 1.40 [95% CI, 1.03–1.91]; p-value=0.033)] and PCI [12.1% in lower and 16.6% in higher (HR, 1.45 [95% CI, 1.03–2.05]; p-value=0.035)]. However, the groups did not differ significantly in the incidence of cardiac death and MI. Effects of a higher average BDNF methylation at baseline on MACE outcomes after adjustment for baseline characteristics cluster are summarised in online Table S4. Considering the crude associations (1st row), the effect of BDNF methylation on MACE was

substantially reduced by depression characteristics (Model 3) but not by other characteristics (Models 2, 4, and 5).

Effects according to depression comorbidity and treatment

Baseline *BDNF* methylation average percentages were significantly higher in patients with depressive disorder (mean, 39.5–39.6) than in those without (mean, 36.9) (p-value<0.001), but similar among the three groups of depressive patients (p-value=0.990). Incidences of the composite MACE were 31.5% (186/591) in patients without depression, 42.5% (54/127) in depressive patients on escitalopram, 54.7% (70/128) in depressive patients on placebo and 59.3% (73/123) in depressive patients on CAU. Significant differences were found between patients with and without depression (p-value<0.001), and among patients with depression (p-value=0.022). Effects of average *BDNF* methylation values on the incidences of MACE according to depression comorbidity and treatment status are summarised in Table 2. In patients without depression, a higher average *BDNF* methylation value was significantly associated with the incidences of composite MACE, all-cause mortality and cardiac death. In depressive patients on placebo, a higher average *BDNF* methylation value was significantly associated with the incidences of composite MACE and MI. In depressive patients on CAU, a higher average *BDNF* methylation value was significantly associated with the incidences of composite MACE and all-cause mortality. However in depressive patients on escitalopram, *BDNF* methylation average status was not associated with any MACE outcomes. The *BDNF* methylation x depressive status multiplicative interaction terms were statistically significant only for the cardiac death outcome. Results on additional sensitivity analysis using the methylation percentages as a continuous variable are summarized in the online Table S5. The strengths of the associations were not substantially changed.

Effects of individual *BDNF* methylation values on the occurrence of MACE

Effects of *BDNF* methylation individual values on the occurrence of MACE are summarised in online Tables S6–S10. Higher *BDNF* methylation percentages at CpG3, CpG7 and CpG9 were significantly associated with the incidence of composite MACE; a higher *BDNF* methylation percentage at CpG9 was significantly associated with the incidence of all-cause mortality; and a higher *BDNF* methylation percentage at CpG3 was significantly associated with the incidences of MI and PCI.

Discussion

The principal finding from this cohort study was that higher *BDNF* methylation status measured at acute phase was significantly associated with worse long-term prognosis of ACS, independent of potential confounding factors. This association was significant in patients without depression, as well as in depressive patients on placebo or CAU, but not in depressive patients treated with escitalopram.

Several mechanisms might explain the significant association between higher *BDNF* methylation and worse prognosis of ACS. In animals, BDNF is an endothelial cell survival factor that plays an essential role in stabilising intramyocardial vessels by stimulating angiogenesis, regulating vascular flow and promoting the revascularisation of ischemic tissue (Donovan et al., 2000; Kermani et al., 2005). In humans, lower blood BDNF levels are associated with higher risk and worse prognosis of cardiovascular disease (Kaess et al., 2015; Manni et al., 2005; Fukushima et al., 2015). Given that BDNF release is reduced in individuals with higher methylation percentages (Martinowich et al., 2003), restorative angiogenic actions against ischemic injury may be impaired in such patients, thereby increasing the risks of recurrence and death. Although our findings have not been reported previously in ACS, similar results were reported in a post-stroke cohort, in which a higher *BDNF* promoter methylation status was independently associated with 1 year stroke functional outcomes, specifically, worsening of physical disability and cognitive function (Kim et al., 2012). In addition, this result supports the existing literature on the epigenetic effects of other genes on the risk or prognosis of ACS (Huica et al., 2011; Peng et al., 2014; Jia et al., 2013; Yang et al., 2016; Breitling et al., 2012).

The *BDNF* methylation sites investigated in this present study are associated with depressive disorder in the general population (Devlin et al., 2010) and in physical disorders

including ACS (Kim et al., 2015b) and stroke (Kim et al., 2013). There is ample evidence that comorbid depression is associated with poor outcomes of ACS, including increased mortality and nonfatal events (Lespérance et al., 2002). Moreover, the effect of BDNF methylation on MACE was substantially reduced by depression characteristics rather than other socio-demographic or cardiac characteristics (see online Table S4). Therefore, it is reasonable to hypothesise that the associations between *BDNF* methylation and ACS prognosis may be influenced by depression status. However, the association remained significant after adjustment for depression severity (baseline BDI scores). Furthermore, the association was significant not only in patients without depression, but also in depressive patients on placebo and CAU, although the methylation percentages were significantly higher in patients with depression than in those without. In addition, pro-inflammatory cytokines have influenced both ACS and depression (Lespérance et al., 2004), and its effects were mediated by BDNF (Calabrese et al., 2014) The BDNF methylation value was significantly associated with serum IL6 levels (see Table 1), but its effect on ACS prognosis was independent of IL6 levels. *BDNF* methylation status was also associated with unmarried marital and unemployed status, and a family history of depression at baseline, which could be considered as other evidence of epigenetic changes. However, these characteristics had little influence on the associations between BDNF methylation and MACE outcomes. These observations imply that the effect of BDNF methylation status on prognosis of ACS is related to angiogenic activity, rather than being mediated by baseline depression or inflammatory status as well as behavioural or historical characteristics.

It is noteworthy that *BDNF* methylation status was not associated with prognosis of ACS in depressive patients treated with escitalopram. Successful antidepressant treatment responses are associated with elevated peripheral BDNF levels in patients with major depressive disorder (Sen et al., 2008; Molendijk et al., 2011). One possible mechanism for the

association observed in this study is phosphorylation of methyl-CpG-binding protein 2 (MeCP2), which acts as an repressor of gene expression, followed by dissociation of MeCP2 from DNA (Hutchinson et al., 2012). Because *BDNF* methylation is a prerequisite for specific MeCP2 binding, it is possible that escitalopram dissociated MeCP2 from DNA and increased the expression of *BDNF*, thereby counteracting the risk of worse cardiac outcomes. However, we cannot confirm these assumptions because *BDNF* methylation was not re-assayed at follow-up. In the sample randomised into the EsDEPACS trial, escitalopram was more effective at treating depression (Kim et al., 2015a) and associated with better cardiac outcomes than placebo (Kim et al., 2018b). Therefore, it is also possible that successful depression treatment with escitalopram may promote better health behaviour, such as increased hospital engagement and treatment adherence (Smolderen et al., 2017), which are closely related to ACS prognosis.

Average *BDNF* methylation percentages were the primary values analysed in this study, and this approach has been repeatedly used in other studies (Lubin et al., 2008; Kelle et al., 2010). With respect to the individual CpG sites, higher *BDNF* methylation percentages at CpG3, CpG7 and CpG9 predicted worse prognosis of ACS. Because these associations were weaker than the association with average values, future replications are needed to confirm these findings.

As the first evaluation of these questions, this study has several strengths, and also benefits from a unique prospective observational and randomised placebo-controlled interventional study design. Participants were recruited at baseline consecutively from among all eligible patients with a recent ACS, thereby decreasing the risk of error arising from heterogeneous examination times and increasing the sample homogeneity. Depressive disorder was ascertained using a structured diagnostic interview, and all measurement methods for psychiatric and cardiovascular characteristics were well validated. Multiple

covariates were considered in the analyses. Recruitment was carried out at a single site, potentially limiting the generalisability of the present findings, but representing an advantage in terms of consistency of evaluation and treatment of patients. One important limitation of this study is that we investigated methylation status in only one CpG island in the *BDNF* gene, although this area has been repeatedly evaluated in previous epigenetic research on depression (Fuchikami et al., 2011; Roth et al., 2009; Devlin et al., 2010; Kang et al., 2015). Another source of limitation was attrition in the recruitment process: methylation analysis was only possible in 84% of the baseline sample. However, baseline demographic and clinical characteristics did not differ between patients for which this information was or was not available. Moreover, long-term follow-up data on MACE are completely obtained for the primary analysis. The study hypotheses were based on prior studies, but the study results could not provide mechanistic evidences, which need future studies. Finally, those taking antidepressants or having a history of neuropsychiatric illnesses were excluded for the EsDEPACS trial, which may represent less severe depression pathologies in these treatment groups and therefore limit the generalizability. However, scores on BDI were significantly higher in EsDEPACS allocated group compared to the CAU group (online Table S1).

In conclusion, higher *BDNF* methylation status at acute phase of ACS was associated with worse long-term prognosis, independent of potential confounders and irrespective of baseline depressive status. *BDNF* methylation tests might have clinical utility in screening for epigenetic susceptibility to identify at-risk groups for recurrence or ACS-related mortality; therefore, this status represents a promising prognostic biomarker in these patients. Escitalopram reduced the negative effect of higher *BDNF* methylation on ACS prognosis. Future studies with other antidepressants, or in non-depressive subjects, could elucidate the exact mechanism. Our results suggest that drugs capable of regulating *BDNF* methylation, should they be developed, could improve prognosis of ACS.

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Role of the sponsor

The funders had no role in study design and conduct of the study; in the collection, management, analysis, and interpretation of data; in the preparation, review, or approval of the manuscript; and in the decision to submit the manuscript for publication.

Author Contributions

Drs J-M Kim and Yoon had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: J-M Kim, Jeong, Yoon.

Acquisition, analysis, or interpretation of data: J-M Kim, Stewart, J-W Kim, Kang, Lee, S-Y Kim, S-W Kim, I-S Shin, Hong, Ahn, Jeong, Yoon.

Drafting of the manuscript: J-M Kim, Stewart.

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Administrative, technical, or material support: J-M Kim, J-W Kim, Kang, S-W Kim, I-S Shin, Hong, Ahn.

Study supervision: J-M Kim, Jeong, Yoon.

Conflict of interest

The authors have no conflict of interest to disclose.

ETHICAL STATEMENT:

The study was performed in accordance with the Declaration of Helsinki.

The study was approved by the institutional review board of Chonnam National University Hospital in Korea.

All procedures were carried out with the adequate understanding and written consent of the subjects.

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Figure 1. Flow diagram for the recruitment process.

Figure legends:

ACS: acute coronary syndrome; BDI: Beck Depression Inventory; MINI: Mini-International Neuropsychiatric Interview. K-DEPACS: Korean DEPression in Acute Coronary Syndrome; EsDEPACS: Escitalopram for DEPression in Acute Coronary Syndrome.

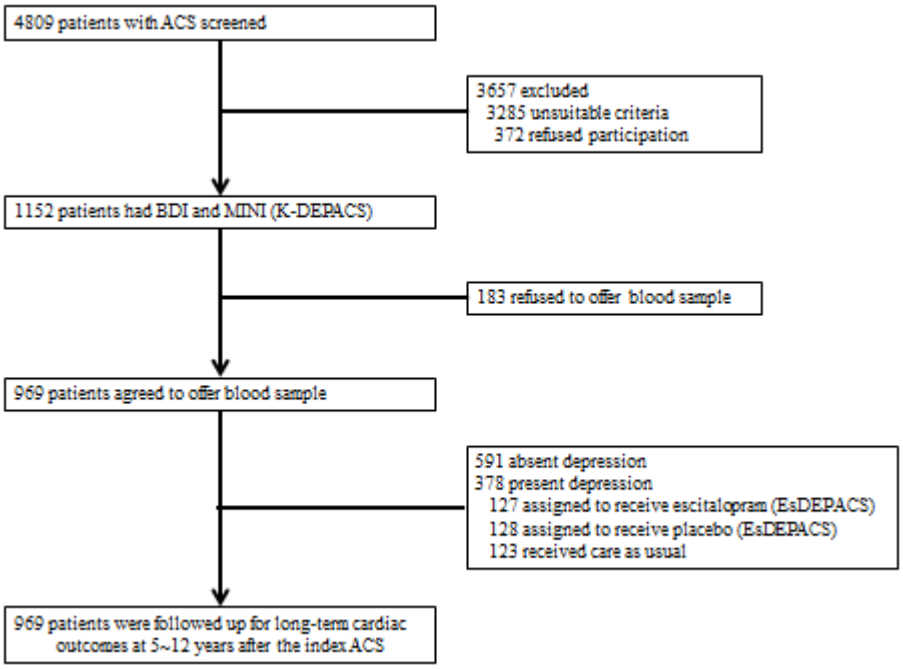

Figure 2. Cumulative incidence (%) of composite major adverse cardiac event (MACE), by average *BDNF* methylation at baseline.

Figure legends:

Hazard ratio (95% confidence interval) [HR (95% CI)] was estimated adjusted for age, education, marital status, employment, Beck Depression Inventory scores, family history of depression, hypertension, diabetes, hypercholesterolemia, obesity, smoking, previous and family history of acute coronary syndrome (ACS), ACS diagnosis, Killip class, left ventricular ejection fraction, and serum levels of troponin I, creatine kinase-MB, and interleukin-6 at baseline.

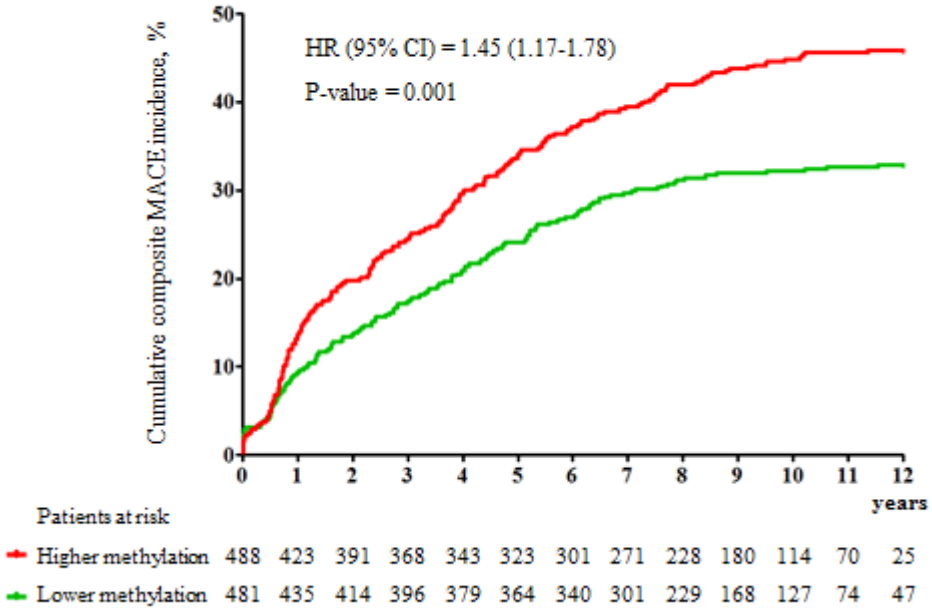

Figure 3. Cumulative incidence (%) of individual major adverse cardiac event (MACE), by average *BDNF* methylation at baseline.

Figure legends:

Hazard ratio (95% confidence interval) [HR (95% CI)] was estimated adjusted for age, education, marital status, employment, Beck Depression Inventory scores, family history of depression, hypertension, diabetes, hypercholesterolemia, obesity, smoking, previous and family history of acute coronary syndrome (ACS), ACS diagnosis, Killip class, left ventricular ejection fraction, and serum levels of troponin I, creatine kinase-MB, and interleukin-6 at baseline.

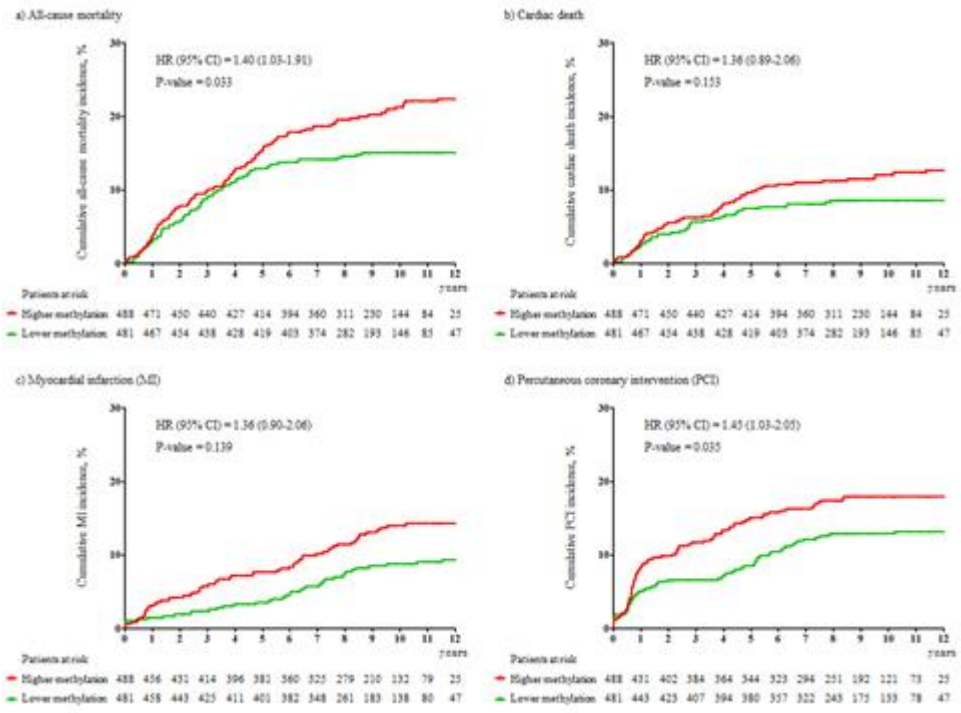

Table 1. Baseline characteristics by average *BDNF* methylation 2 weeks after acute coronary syndrome (ACS).

	Lower methylation (N=481)	Higher methylation (N=488)	Statistical coefficient	P-value*
Socio-demographic characteristics				
Age, mean (SD) years	58.1 (11.1)	58.3 (11.2)	t=-0.331	0.741
Gender, N (%) female	125 (26.0)	144 (29.5)	χ^2=1.497	0.221
Education, mean (SD) years	10.0 (4.6)	9.7 (4.7)	t=+1.048	0.295
Unmarried marital status, N (%)	59 (12.3)	82 (16.8)	χ^2=4.011	**0.045**
Living alone, N (%)	45 (9.4)	47 (9.6)	χ^2=0.021	0.884
Housing, N (%) rented	70 (14.6)	80 (16.4)	χ^2=0.627	0.428
Currently unemployed, N (%)	162 (33.7)	206 (42.2)	χ^2=7.489	**0.006**
Depression characteristics, N (%)				
BDI, mean (SD) score	8.6 (8.1)	11.4 (8.9)	t=-5.213	**<0.001**
Depression comorbidity and treatment				
No depression	341 (70.9)	250 (51.2)	χ^2=39.59	**<0.001**
Depression on escitalopram	48 (10.0)	79 (16.2)		
Depression on placebo	48 (10.0)	80 (16.4)		
Depression on care as usual	44 (9.1)	79 (16.2)		
Previous depression	16 (3.3)	18 (3.7)	χ^2=0.094	0.759
Family history of depression	6 (1.2)	17 (3.5)	χ^2=5.227	**0.022**
Cardiac risk factors, N (%)				
Hypertension	235 (48.9)	223 (45.7)	χ^2=0.970	0.325
Diabetes mellitus	89 (18.5)	102 (20.9)	χ^2=0.881	0.348

Hypercholesterolemia	233 (48.4)	253 (51.8)	χ^2=1.122	0.289
Obesity	210 (43.7)	255 (42.0)	χ^2=0.270	0.604
Current smoker	191 (39.7)	175 (35.9)	χ^2=1.526	0.217
Previous ACS	18 (3.7)	21 (4.3)	χ^2=0.197	0.657
Family history of ACS	16 (3.3)	15 (3.1)	χ^2=0.050	0.823
Current cardiac status				
ACS diagnosis, N (%)				
Myocardial infarction	351 (73.0)	363 (74.4)	χ^2=0.249	0.618
Unstable angina	130 (27.0)	125 (25.6)		
Killip class >1, N (%)	92 (19.1)	76 (15.6)	χ^2=2.134	0.144
LVEF, mean (SD) %	62.0 (11.2)	60.4 (11.4)	t=+2.204	**0.027**
Troponin I, mean (SD) mg/dL	9.8 (14.5)	10.0 (15.3)	t=-0.193	0.847
CK-MB, mean (SD) mg/dL	17.8 (35.5)	17.0 (38.9)	t=+0.343	0.732
Interleukin-6, mean (SD) pg/mL	23.9 (17.1)	27.2 (19.9)	t=-2.255	**0.024**

*p-values using t-tests or χ^2 tests as appropriate.

BDI, Beck Depression Inventory; LVEF, left ventricular ejection fraction; and CK-MB, Creatine kinase-MB.

Table 2. Associations of a higher average *BDNF* methylation at baseline with long-term cardiac outcomes in patients with acute coronary syndrome (ACS), by depression comorbidity and randomised treatment status. Data are hazard ratios (95% confidence intervals) [HRs (95% CIs)].

	Absent depression (N=591)	Depression on escitalopram (N=127)	Depression on placebo (N=128)	Depression on care as usual (N=123)	P-value for interaction
Major adverse cardiac events	1.39 (1.01-1.90)*	1.00 (0.51-1.95)	1.72 (1.02-3.02)*	1.53 (1.01-2.61)*	0.591
All-cause mortality	1.72 (1.08-2.70)†	0.86 (0.36-2.07)	1.47 (0.45-4.77)	1.89 (1.00-3.99)*	0.624
Cardiac death	2.20 (1.01-4.20)*	0.24 (0.05-1.11)	1.02 (0.50-2.91)	1.94 (0.53-7.11)	0.012
Myocardial infarction	0.88 (0.48-1.62)	6.83 (0.28-67.38)	3.43 (1.00-12.35)*	1.17 (0.34-3.99)	0.103
Percutaneous coronary intervention	1.33 (0.81-2.18)	0.76 (0.22-2.67)	1.39 (0.53-3.62)	1.37 (0.53-3.53)	0.882

HR (95% CI) was estimated adjusted for age, education, marital status, employment, Beck Depression Inventory scores, family history of depression, hypertension, diabetes, hypercholesterolemia, obesity, smoking, previous and family history of ACS, ACS diagnosis, Killip class, left ventricular ejection fraction, and serum levels of troponin I, creatine kinase-MB, and interleukin-6 at baseline.

*p-value<0.05; † p-value<0.01.

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